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A multicenter, open-label, prospective, randomized, dose-ranging pharmacokinetic study of the anti-TNF-α antibody afelimomab in patients with sepsis syndrome

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Abstract *Objective:* To investigate the pharmacokinetics and safety of afelimomab, a murine antibody fragment against human tumor necrosis factor (TNF)-α in patients with sepsis.
Design: Multicenter, randomized, open-label, placebo-controlled phase I/II clinical trial.
Setting: Intensive care units of six academic medical centers in the United States.
Patients: Forty-eight patients with a clinical diagnosis of sepsis who received standard supportive care and antimicrobial therapy.
Interventions: Patients received 0.3, 1.0, or 3.0 mg/kg afelimomab or placebo intravenously over 20 min. Three patients in each dose group received single doses; the remaining nine patients in each group received multiple (nine) doses at 8-h intervals over 72 h.
Measurements and main results: Afelimomab appeared safe and well tolerated. Single- and multiple-dose kinetics were predictable and dose related. The elimination half-life was 44.7 h. Afelimomab treatment resulted in increased serum concentrations of TNF (includes TNF-antibody complexes) and decreased serum interleukin-6 concentrations, whereas no discernible trends were observed in placebo-treated patients. There was no significant treatment effect on 28-day mortality as was expected given the small number of patients. However, overall mortality was significantly ($p = 0.001$) associated with baseline interleukin-6 concentration. All patients experienced adverse events, but the vast majority were considered unrelated to the study drug and demonstrated no apparent relationship to afelimomab dose. Although 41% of patients developed human anti-murine antibodies, there were no clinical sequelae.
Conclusions: Multidose therapy with afelimomab was safe, well tolerated, and had predictable linear kinetics. A large randomized trial comparing afelimomab to placebo in patients with well defined sepsis has recently been completed.

Keywords Afelimomab · MAK 195F · Anti-TNF-α antibody · Interleukin-6 · Sepsis · Septic shock

Introduction

Sepsis and septic shock remain major causes of morbidity and mortality in spite of the advances in antibiotic therapy and medical technology. The sepsis syndrome develops in more than 500,000 patients in the United States each year, and the incidence appears to be increasing despite a growing armamentarium of antibiotics and an enhanced knowledge of the pathophysiological processes involved [1]. Furthermore, surveys have shown a worrisome increase in resistance to important antibiotics [2, 3]. Based on analyses of large intensive care databases, the overall mortality for patients critically ill with sepsis ranges from 20% to 60% [4, 5, 6]. Many of the clinical sequelae and the high mortality rates associated with infection are due, at least in part, to the local and systemic inflammatory responses of the host triggered by the invading micro-organisms [7]. These host responses can result in the severe tissue damage and life-threatening physiological perturbations seen in sepsis and are not being adequately treated with current antibiotic therapy and general supportive care.

Experimental evidence suggests that the cytokines interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6 are the primary early mediators of these host responses [8, 9, 10, 11]. Elevated plasma TNF-α concentrations can be seen in cases of either Gram-negative or Gram-positive bacteremia, [10, 11], and several lines of experimental evidence have suggested that TNF-α is a particularly important mediator of sepsis. Following administration of live or heat-killed bacteria or endotoxin, TNF-α is present in the systemic circulation, [12, 13] and direct administration of TNF-α produces many of the physiological and laboratory changes associated with severe sepsis [14, 15, 16]. Antibodies against TNF-α have demonstrated a protective effect in animal models of severe sepsis [17, 18]. Recently Mira et al. [19] reported finding a polymorphism within the TNF-α gene promoter, associated with enhanced TNF-α production and negative outcome in some severe infections.

Because of the persuasive evidence that TNF-α is an important mediator of septic shock, therapy that includes TNF-α neutralization is a compelling approach. Several clinical trials in septic patients with antihuman TNF-α monoclonal antibodies or soluble TNF receptor constructs have recently been reported [20, 21, 22, 23, 24, 25]. Most trials have been unable to demonstrate a clinical benefit, but there has been some indication that anti-TNF-α therapy benefits those septic patients with the worst prognosis [21, 26]. In addition, no overt toxicity from anti-TNF-α monoclonal antibodies has been observed, and thus this anticytokine immunotherapeutic strategy remains promising.

The murine monoclonal $F(ab')_2$ antibody fragment afelimomab has been developed to avidly bind to and neutralize human TNF-α [27]. The fragment rather than the whole antibody was chosen to reduce potential immunogenicity, improve tissue penetration, and minimize interaction with Fc receptors. Afelimomab effectively blocks the biological effects of human TNF-α in vitro [27] and is highly effective in animal models of sepsis [18, 28]. A phase I study demonstrated the safety of repeated infusions of up to 3 mg/kg per dose in septic patients [29]. The primary objective of this phase I/II study was to determine the pharmacokinetics of intravenous afelimomab at three doses (0.3, 1.0, and 3.0 mg/kg) in patients with sepsis syndrome. Also evaluated were the safety, immunogenicity, serum TNF-α and IL-6 concentrations, and mortality in both afelimomab- and placebo-treated patients.

Materials and methods

This multicenter, open-label, randomized placebo-controlled study was conducted in six centers in the United States. The protocol was approved by the local ethics committees, and written informed consent was obtained for each patient from either the patient or a responsible relative. The study was conducted in accordance with the Helsinki Declaration as amended in Tokyo, Venice, and Hong Kong.

Male and nonpregnant female patients at least 18 years of age admitted to the intensive care unit with a clinical diagnosis of sepsis were enrolled in the study. Eligible patients had to fulfilled all five entry criteria within 24 h of one another: (a) clinical evidence of sepsis; (b) fever (≥38.0 °C) or hypothermia (≤35.6 °C); (c) tachycardia (≥90 beats/min) in the absence of β-blockade; (d) tachypnea (≥20 breaths/min) or respiratory distress requiring mechanical ventilation; (e) either hypotension (systolic blood pressure ≤90 mmHg or a sustained drop in systolic blood pressure ≥40 mmHg) in the absence of antihypertensive agents and vasopressors, or evidence of systemic toxicity or poor end-organ perfusion. Patients with systemic toxicity or poor end-organ perfusion were required to have two or more of the following: metabolic acidosis (pH ≤7.3), arterial hypoxia (pO_2 ≤75 mmHg) in those without overt pulmonary disease, plasma lactate concentration above the normal range of the testing laboratory, acute renal failure (oliguria with urine output ≤0.5 ml/kg per hour for 1 h or longer), unexplained coagulation abnormality (prothrombin time ≥1.2 or partial thromboplastin time ≥1.2× control value) within the prior 24 h, unexplained platelet depression (≤100,000 platelets/ml or a decrease by ≥50% from baseline) within the prior 24 h, acute deterioration of mental status, and a cardiac index less than 4.0 l min^{-1} m^{-2} with a systemic vascular resistance greater than 800 dynes s^{-1} cm^{-5}. Patients were excluded if they had received an investigational agent within the prior 30 days, had previously received any murine monoclonal antibody, had received oral or parenteral steroids within the past 7 days, were HIV positive, or were septic following major burns or organ transplant. Patients could be withdrawn from the study for any reason but had to be withdrawn if they developed intolerable adverse experiences or any exclusion criteria.

Qualified patients were randomized to receive either placebo or one of 3 doses (0.3, 1.0, and 3.0 mg/kg) of the anti-TNF-α antibody afelimomab (also known as MAK 195F, Knoll, Ludwigshafen, Germany). The first three patients randomized to each of the four treatment groups received a single intravenous dose of study drug infused over 20 min. The subsequent nine patients in each

Table 1 Characteristics of multiple-dose patients at baseline (APACHE Acute Physiology and Chronic Health Evaluation, *TNF* tumor necrosis factor, *IL* interleukin)

Characteristic	Placebo ($n = 9$)	Afelimomab			All patients ($n = 36$)
		0.3 mg/kg ($n = 9$)	1.0 mg/kg ($n = 9$)	3.0 mg/kg ($n = 9$)	
Age (years)	67.2 ± 18.9	57.9 ± 15.8	60.2 ± 12.3	55.0 ± 21.4	60.1 ± 17.3
Sex (male/female)	5/4	6/3	4/5	3/6	18/18
Race (white/black)	8/1	6/3	9/0	8/1	31/5
Weight (kg)	72.2 ± 11.4	74.2 ± 14.6	79.6 ± 23.4	102.8 ± 40.3	82.2 ± 27.0
Severity of underlying disease (%)					
Nonfatal/none	77.8	55.6	66.7	77.8	69.4
Ultimately fatal	22.2	33.3	33.3	22.2	27.8
Rapidly fatal	0	11.1	0	0	2.8
Shock present (%)	88.8	66.7	100	66.7	80.6
Mechanically ventilated (%)	77.8	66.7	100	77.8	80.6
APACHE II score	29.3 ± 9.81	25.1 ± 7.47	27.4 ± 7.27	22.3 ± 10.17	26.0
TNF-α (pg/ml)					
Median	37.0	83.8	40.3	15.6	32.2
Range	≤15.6–392.0	≤15.6–1454.0	≤15.6–69.7	≤15.6–79.1	≤15.6–1454.0
IL-6 (pg/ml)					
Median	8599	3466	876	524	1537
Range	46–1.2×10^6	92–3.9×10^6	36–1.4×10^5	33–4.5×10^3	33–3.9×10^6

group received study drug every 8 h for a total of nine doses, each infused over 20 min. In addition, patients received antibiotic therapy as well as aggressive resuscitative, diagnostic, and supportive care as determined by their treating physicians.

Blood samples were drawn from each single-dose patient at 0, 5 min, and 0.5, 1, 6, 12, 24, 48, 72, 120, and 168 h postinfusion for determination of afelimomab concentrations. Multiple-dose patients had blood samples taken at 0, 5 min, and 0.5, 1, 6, and 12 h following the first infusion and at 1, 6, 12, 24, 48, 72, 120, and 168 h following the last infusion for determination of afelimomab concentrations. The serum concentration of afelimomab was determined by enzyme-linked immunosorbent assay (ELISA) with a 3.0 ng/ml lower limit of quantitation. The primary pharmacokinetic variables were the model-fitted parameters for systemic serum afelimomab concentrations, including the peak serum concentration (C_{max}), area under the serum concentration-time curve (AUC), clearance rate (CL), steady-state volume of distribution (V_{ss}), and elimination half-life ($t_{1/2}$). Area under the moment curve (AUMC) and mean residence time (MRT) were also determined.

Serum concentrations of TNF-α and IL-6 were determined in blood samples taken from multiple-dose patients at 0, 0.5, 1, 6, 12, 24, 48, 72, 120, and 168 h. Blood samples were taken just prior to afelimomab infusion. TNF-α and IL-6 concentrations were determined using the Medgenix TNF-α ELISA and IL-6 ELISA kits (BioSource Europe, Fleurus, Belgium), respectively, which had an estimated 15.6 pg/ml lower limit of quantitation for both cytokines.

Patients were followed for 28 days after the initial treatment. Patients were evaluated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score on enrollment and on days 1, 2, and 3 of the study, and survival was assessed at 28 days after study initiation. All adverse clinical changes from the patient's pretreatment condition (including intercurrent illness) were recorded throughout the 28-day study. Safety parameters were collected throughout the study, including vital signs (temperature, blood pressure, and heart rate), respiratory parameters (respiratory rate, FIO_2, ventilatory mode, tidal volume, minute volume, and positive end-expiratory pressure/continuous positive airway pressure), arterial blood gases (pH, pCO_2, pO_2), urine output, and clinical hematology and biochemistry variables (hemoglobin, hematocrit, cell counts, prothrombin time, partial thromboplastin time, fibrinogen, electrolytes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, glucose, albumin, C-reactive protein, lactate, uric acid, urea, and creatinine). Plasma samples were drawn at 0, 14, 21, and 28 days for qualitative assessment of human anti-mouse antibodies (HAMA) using the ImmuStrip HAMA IgG kit (Immunomedics, Morris Plains, N.J., USA). Twelve-lead electrocardiograms were taken at enrollment and on days 1 and 4 of the study.

Statistical analysis

All randomized patients who received trial medication were included in the analyses. Pharmacokinetic parameters for the single-dose patients were analyzed descriptively, and data from multiple-dose patients were analyzed separately.

Pharmacokinetic analyses were performed using WinNonlin Professional version 1.5, model 10 (two-compartment intravenous infusion input) or model 19 (three-compartment intravenous infusion input) selected by the best fit of the data. Actual (relative to start of infusion), rather than protocol-specified times of blood collection were used in all pharmacokinetic calculations unless unavailable. All skewed continuous variables were (natural log) transformed to better approximate a normal distribution. The relationships between AUC and dose and between log-transformed baseline TNF-α and IL-6 concentrations were assessed by regression analysis.

Efficacy and safety variables were analyzed descriptively. Because of the small number of patients in each treatment group, data were collapsed across centers and statistically significant differences were not expected. Testing was limited to efficacy variables and was intended to identify trends and generate hypotheses.

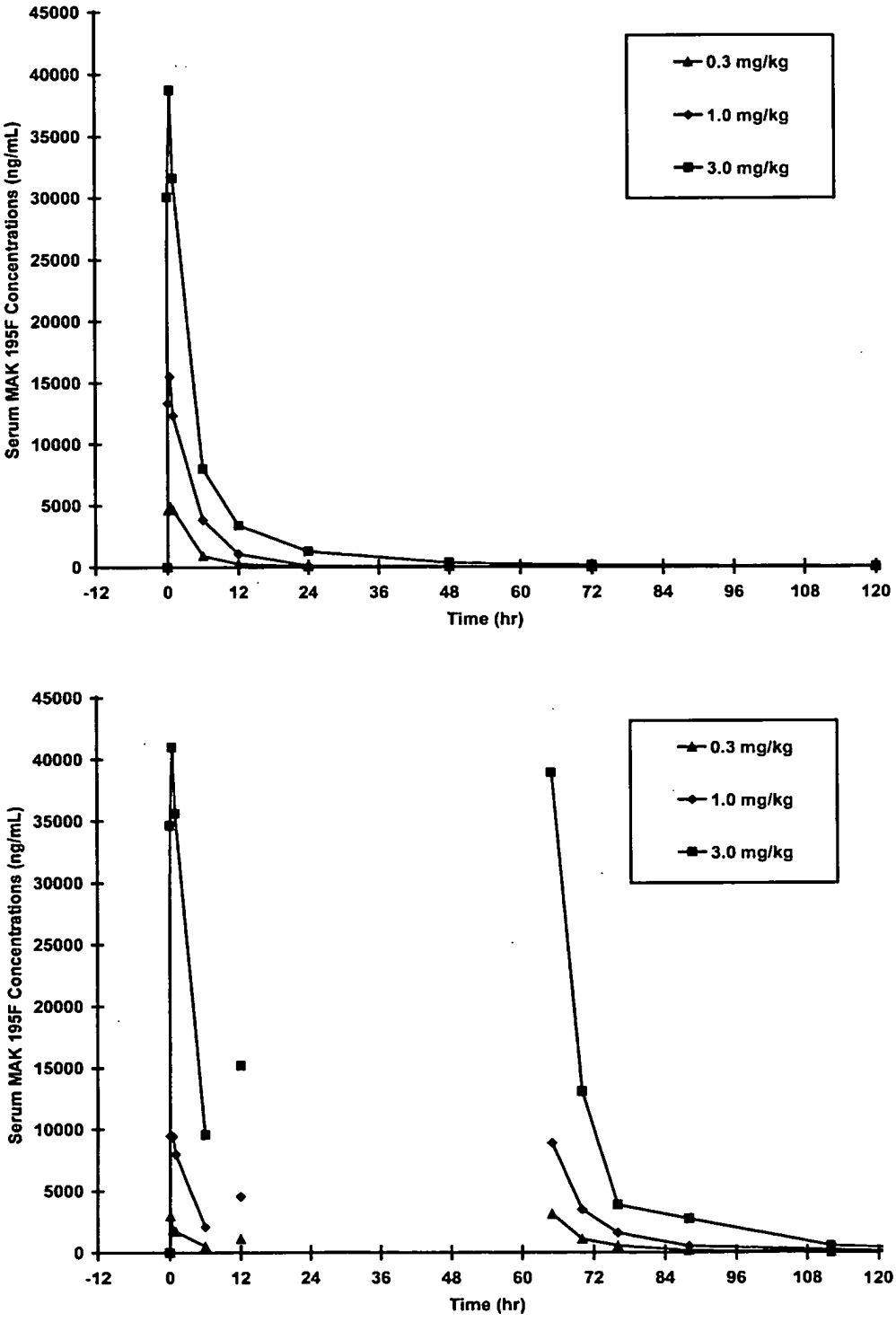

Fig. 1 Mean serum afelimomab profiles following single *(above)* and multiple *(below)* infusions

The analyses were performed using SAS version 6.08 (SAS Institute, Cary, N.C., USA).

The influence of baseline parameters on the probability of 28-day mortality was investigated with a logistic regression model. The dependent variable was mortality on day 28; treatment was included in the model a priori. Further selection among the factors considered clinically relevant was based on an initial univariate analysis. Factors included were those achieving a p value of 0.15 in a forward selection process. There were no α-corrections for multiple comparisons since all results were descriptive.

Table 2 Mean pharmacokinetic parameters for afelimomab (*AUC* area under the curve, *AUMC* area under the moment curve, C_{max} maximum serum concentration, *MRT* mean residence time, $t_{1/2}$ half life, *CL* clearance, V_{ss} volume of distribution at steady state)

Parameters	0.3 mg/kg (n = 6)	1.0 mg/kg (n = 9)	3.0 mg/kg (n = 9)
$AUC_0 \rightarrow \infty$ (ng ml^{-1} h^{-2})	16,039 ± 6,962	48,297 ± 19,289	169,671 ± 76,660
AUMC (ng ml^{-1} h^{-2})	213,629 ± 139,317	490,975 ± 397,213	1,454,965 ± 1,014,389
C_{max} (ng ml^{-1})	3,246 ± 922	12,817 ± 3,890	48,460 ± 19,154
CL (ml min^{-1} kg^{-1})	0.41 ± 0.30	0.40 ± 0.15	0.36 ± 0.19
V_{ss} (l kg–1)	0.259 ± 0.108	0.191 ± 0.073	0.182 ± 0.161
MRT (h)	12.2 ± 5.1	8.9 ± 4.3	8.3 ± 4.7
$t_{1/2}$, terminal (h)	63.6 ± 45.2	35.0 ± 14.0	56.3 ± 31.7

Results

A total of 48 patients were enrolled in the study, including 12 who received a single dose of study drug and 36 who received multiple doses. Multiple-dose patients across treatment groups were generally similar in their demographic characteristics and measures of disease severity, given the small number of patients in each group (Table 1). The population as a whole reflected the severity of illness of septic patients commonly seen in intensive care units. Cytokine (TNF-α and IL-6) concentrations at baseline varied widely among individuals and between treatment groups. All patients received multiple concomitant drugs including antimicrobials and other medications as a part of overall medical support during the study.

Mean serum afelimomab concentrations following either single (Fig. 1A) or multiple infusions (Fig. 1B) appeared to be dose-related. Pharmacokinetic parameters were determined from those multiple-dose patients who had sufficient numbers of blood samples for model fitting (n = 24). In multiple-dose patients, measures of systemic exposure (mean AUC, AUMC, and C_{max}) increased with increasing afelimomab dose (Table 2). The mean AUC appeared to be dose proportional across the three dose groups (0.3, 1.0, and 3.0 mg/kg; r^2 = 0.675). Measures of drug elimination (mean CL, Vss, MRT, and $t_{1/2}$) were comparable across dose groups with no apparent dependence on dose. The mean elimination $t_{1/2}$ for all afelimomab-treated patients was 44.7 h (range 16.5–150.9), with the high variability in part due to the wide range in final sampling times (120–504 h). However, CL and AUC estimations were not affected significantly by the variations in terminal $t_{1/2}$ as the contribution to the AUC from the serum levels beyond 120 h is minimal.

Serum TNF-α and IL-6 concentrations were highly variable both at baseline and throughout the study, and

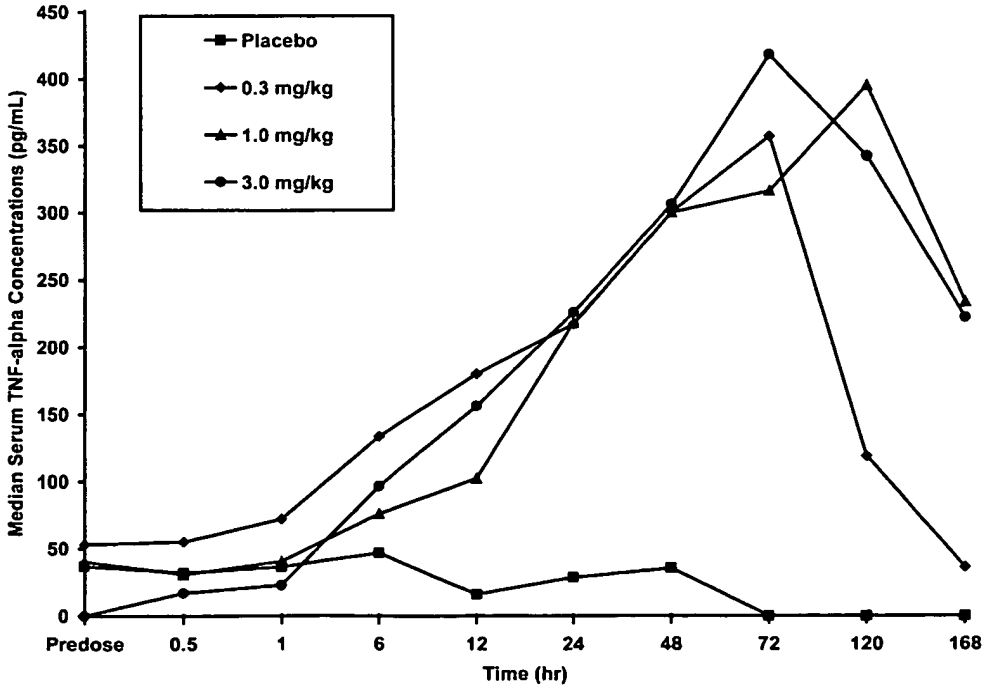

Fig. 2 Median serum TNF-α concentrations in patients receiving multiple doses of afelimomab or placebo

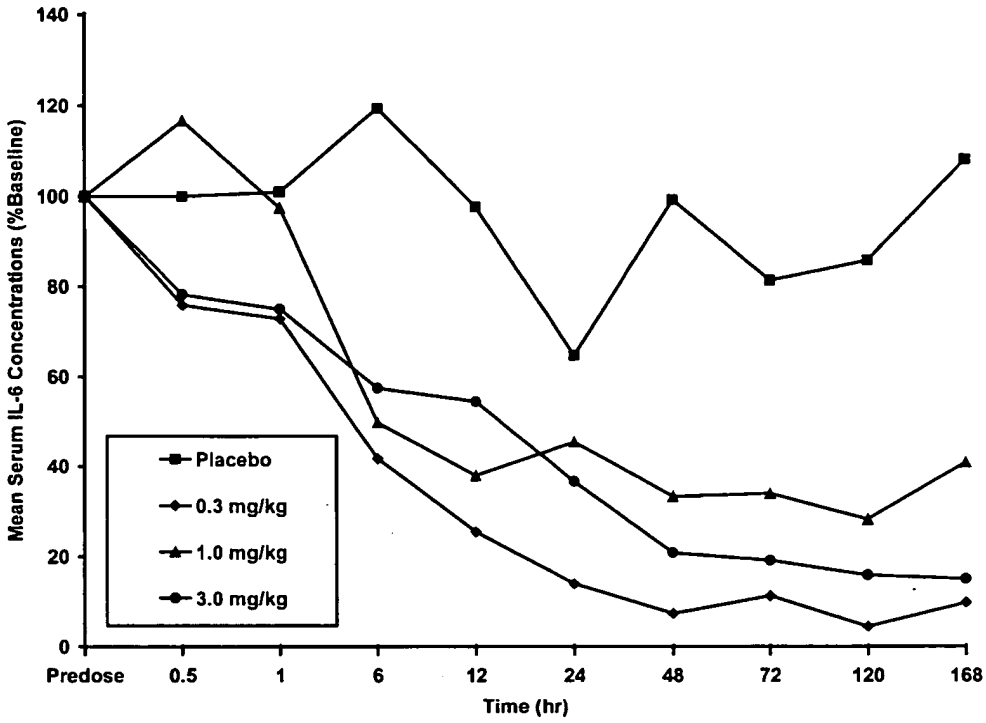

Fig. 3 Mean percentage of baseline serum IL-6 concentrations in patients receiving multiple doses of afelimomab or placebo

log transformation was used to compensate for the skewed distribution. The TNF-α concentration was below the limit of quantitation at baseline in 20 of the 48 (42%) single and multiple-dose patients. However, in all patients with measurable TNF at baseline, there was a weak positive correlation between log transformed baseline TNF-α and IL-6 concentrations ($r^2 = 0.510$). The median serum TNF-α concentration for the placebo group changed little over the 168-h (7 day) observation period, whereas that for all three afelimomab groups increased, beginning at 6 h and continuing until 72–120 h after the first infusion, and declined thereafter (Fig. 2). Median peak TNF-α concentrations were 357, 395, and 418 pg/ml for the 0.3, 1.0, and 3.0 mg/kg dose groups, respectively. There was little difference in TNF-α concentrations between the three groups, with the exception of the return to baseline at 168 h in the lowest afelimomab dose group.

Contrary to TNF-α, median IL-6 concentrations in all treatment groups decreased with time and were less than 100 pg/ml in each group by 168 h. This occurred in the placebo group because of the early deaths (within 12 h) of three patients with the highest IL-6 concentrations (91–1200 μg/ml). Consequently, IL-6 concentrations were analyzed in only those patients with data available for at least 72 h ($n = 30$) and were expressed as percentage of baseline value because of the wide variability between patients. In those patients who received placebo there was no discernible trend in the IL-6 concentration over time (Fig. 3). However, in patients receiving afelimomab serum IL-6 concentrations decreased within the first 12–24 h to between 10% and 40% of baseline, with no apparent relationship to afelimomab dose.

Although this study was not designed or powered to assess the treatment effect on mortality, survival at 28 days following the first drug infusion was recorded for multiple-dose patients. All-cause mortality was highest in the placebo group (56%) and less in the afelimomab-treated groups (33%, 22%, and 22% for the 0.3, 1.0, and 3.0 mg/kg doses, respectively). When patients were stratified by baseline IL-6 concentrations greater than or less than 1000 pg/ml, a differential effect of afelimomab treatment was suggested. Survival curves from patients with baseline IL-6 less than 1000 pg/ml show few deaths in any treatment group (Fig. 4A). However, in patients with baseline IL-6 greater than 1000 pg/ml, mortality rates appeared different among treatment groups (Fig. 4B). All patients receiving placebo and 43% of patients receiving 0.3 mg/kg afelimomab died, but no patients receiving 1.0 mg/kg and only 25% of patients receiving 3.0 mg/kg afelimomab died.

Mortality rates for all multiple-dose patients appeared related to clinical measures of disease severity (i.e., organ failure, presence of shock, APACHE II score, underlying condition) and age. However, the small study population made it impossible to reach definitive conclusions in this regard. When analyzed using a logistic regression model, the log of the baseline IL-6

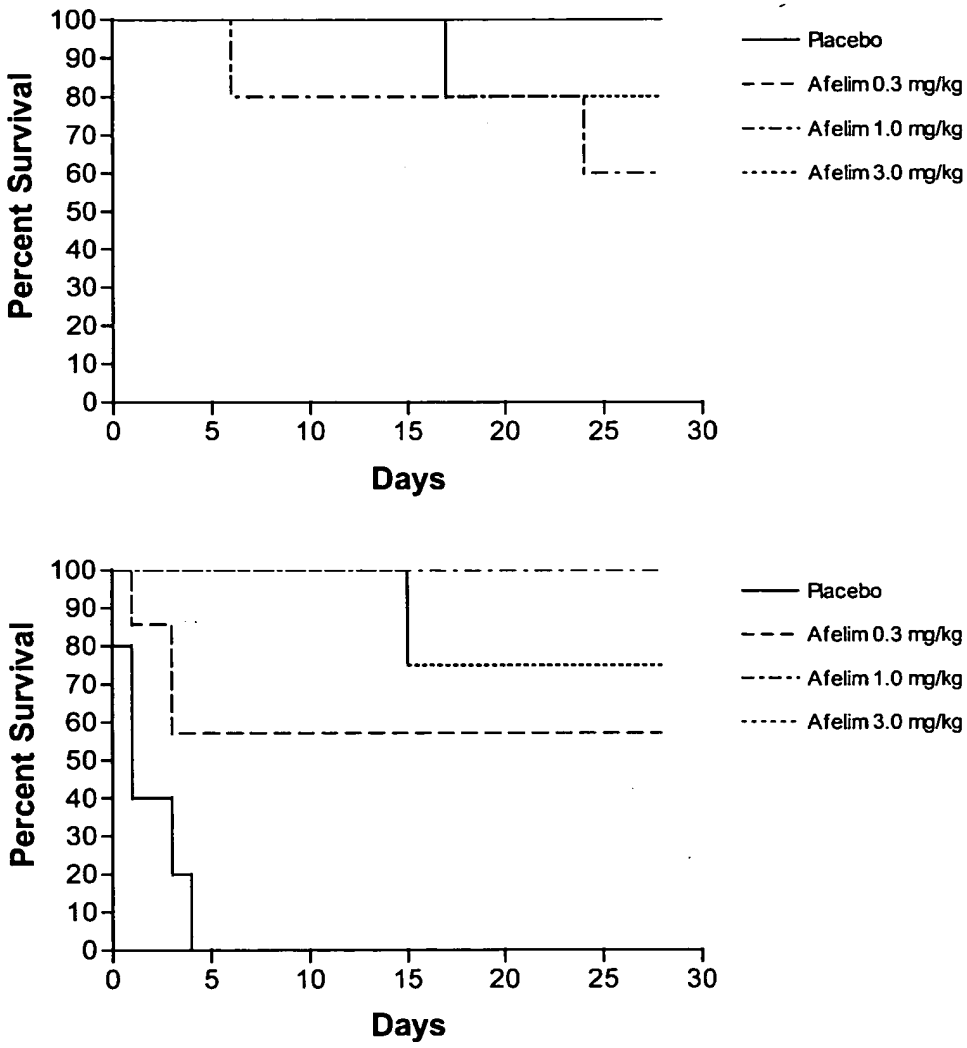

Fig. 4 Kaplan-Meier survival curves for patients receiving multiple doses of afelimomab or placebo stratified by baseline serum IL-6 concentrations < 1000 pg/ml *(above)* or ≥1000 pg/ml *(below)*

concentration was found to be significantly associated with mortality ($p = 0.001$, odds ratio = 1.72). This is reflected in the low 19% overall mortality in multiple-dose patients with baseline IL-6 concentration less than 1000 pg/ml ($n = 16$) and the 45% mortality in those with baseline IL-6 concentration greater than 1000 pg/ml ($n = 20$; Fig. 4).

Safety

All patients experienced adverse events. However, the vast majority of adverse events were judged by investigators to be unrelated to study medication, and no patient had treatment interrupted because of adverse events. The total number of adverse events reported for multiple-dose patients receiving afelimomab were 90, 129, and 92 for the 0.3, 1.0, and 3.0 mg/kg groups, respectively, and 48 for those receiving placebo.

The individual adverse events were of a wide variety expected in this patient population, and their incidence demonstrated no apparent relationship to afelimomab dose and no discernable pattern or relationship to a particular body system (Table 3). Afelimomab was well tolerated overall, and no clinical evidence of afelimomab-related toxicity (i.e., hypotension, arrhythmias, bronchospasm, skin rashes) was observed at any dose. HAMA developed in 11 of the 27 patients (41%) treated with afelimomab in a dose-dependent manner, but no sequelae attributable to the positive HAMA (including serum sicknesslike reactions, rashes, or evidence of hypersensitivity reactions) were demonstrated. The overall incidence of treatment-emergent electrocardiogram changes appeared to be greater in patients in the afelimomab dose groups, but their incidence was not dose dependent. In addition, there were no discernible differences between treatment groups in vital signs, treatment-emergent electrocardiographic changes or

Table 3 Most frequent adverse events (whether or not treatment-related) occurring in ≥20% of patients

	Placebo ($n = 9$)		Afelimomab 0.3 mg/kg ($n = 9$)		Afelimomab 1.0 mg/kg ($n = 9$)		Afelimomab 3.0 mg/kg ($n = 9$)		All afelimomab ($n = 27$)	
Event	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Hypotension	4	44.4	4	44.4	5	55.6	2	22.2	11	40.7
Cardiac arrest	3	33.3	2	22.2	2	22.2	1	11.1	5	18.5
Rash	2	22.2	1	11.1	2	22.2	3	33.3	6	22.2
Hypoxia	2	22.2	1	11.1	4	44.4	0	0	5	18.5
Vomiting	1	11.1	1	11.1	1	11.1	2	22.2	4	14.8
Dyspnea	1	11.1	1	11.1	1	11.1	2	22.2	4	14.8
Pneumothorax	1	11.1	0	0	2	22.2	0	0	2	7.4
Atrial flutter	0	0	0	0	2	22.2	0	0	2	7.4
Pharyngitis	0	0	2	22.2	0	0	0	0	2	7.4
Anxiety	0	0	1	11.1	0	0	2	22.2	3	11.1
Supraventricular extrasystoles	0	0	1	11.1	2	22.2	0	0	3	11.1
Congestive heart failure	0	0	1	11.1	2	22.2	0	0	3	11.1
Abdominal pain	0	0	2	22.2	0	0	1	11.1	3	11.1
Edema	0	0	1	11.1	2	22.2	1	11.1	4	14.8
Generalized edema	0	0	0	0	2	22.2	2	22.2	4	14.8
Peripheral edema	0	0	3	33.3	1	11.1	0	0	4	14.8
Fever	0	0	2	22.2	1	11.1	1	11.1	4	14.8
Nausea	0	0	2	22.2	0	0	2	22.2	4	14.8
Diarrhea	0	0	2	22.2	1	11.1	2	22.2	5	18.5
Ventricular extrasystoles	0	0	2	22.2	2	22.2	1	11.1	5	18.5
Atrial fibrillation	0	0	1	11.1	3	33.3	1	11.1	5	18.5
Supraventricular tachycardia	0	0	0	0	5	55.5	1	11.1	6	22.2

laboratory values. All deaths were ascribed to the ongoing course of the patient's clinical condition.

Discussion

Treatment with single and multiple doses of afelimomab up to 3.0 mg/kg appeared safe and well tolerated in this group of septic patients. Pharmacokinetic parameters that measure systemic exposure appeared to be dose dependent, and those parameters that measure the elimination of the agent had no apparent relationship to dose. There was no indication that the single-dose pharmacokinetics differed from those with multiple dosing, and the linear pharmacokinetics reported here confirm those found in a previous phase I study in septic patients [29].

As would be expected in this critically ill population, adverse events were observed in all patients in each treatment group. However, the majority of the adverse events were considered unrelated to treatment and showed no dose dependency. All deaths were attributable to the progression of sepsis. Although HAMA developed in 41% of patients, there were no apparent clinical sequelae and no evidence of interference with afelimomab clearance or its ability to bind TNF-α. The development of anti-murine antibodies with multiple doses of afelimomab was considerably less frequent than has been reported with single-dose treatments of full-length murine monoclonal antibodies (76–100% of patients) [26, 30]. These data confirm those from previously reported phase I and II studies that had also revealed no indications of intolerance to single- or multiple-doses of afelimomab [21, 29].

Afelimomab binds to a single TNF epitope, neutralizing TNF activity. There was no indication that the increase in total serum TNF observed in afelimomab-treated patients represented an increase in active cytokine. A possible explanation for the rise in TNF concentration was that the Medgenix ELISA recognized several epitopes on TNF-α and thus measured both free and antibody-bound TNF-α. Moreover, the TNF-antibody complex probably had a half-life similar to that of the antibody, rather than the shorter half-life of the cytokine, accounting for the apparent increase in concentration. Consistent with this hypothesis was the return to baseline value by 168 h in the lowest afelimomab-dose group, whereas in the higher dose groups the greater amount of antibody had not yet cleared. These pharmacodynamic findings suggest that under the conditions of the present study 1.0 mg/kg was the minimally effective dose relative to TNF neutralization. Similar increases in TNF concentration have been seen in studies with other anti-TNF antibodies or a TNF receptor construct using the same assay kit or similar methodology [20, 24, 31].

It has been postulated that high serum IL-6 concentrations are inversely correlated with survival [10, 32]. In this study there was a highly significant association between high baseline IL-6 concentration and mortality. Consistent with the role of TNF in stimulating IL-6 production, TNF-α neutralization resulted in a rapid reduction in serum IL-6 concentration regardless of baseline value or afelimomab dose, whereas treatment with placebo had no discernible effect.

Although the concept of TNF neutralization is promising and has been validated in animal models of sepsis, [17, 18] clinical trials with several anti-TNF agents have not yet convincingly demonstrated survival benefit [22, 23, 24]. These disappointing results might be due in part to the inability to accurately define a circumscribed patient population most likely to benefit from anti-TNF therapy [22, 33]. Indeed, the commonly used entry criteria for sepsis trials have been the highly nonspecific cluster of symptoms and signs comprising sepsis syndrome [1].

It is reasonable to speculate that patients with an inappropriate hyperinflammatory response to an infecting organism and elevated levels of TNF would be the group likely to benefit from anti-TNF therapy, whereas those with an appropriate response to the initial insult may not be helped. While concentrations of TNF-α in local tissues may be the best measure of the degree of the proinflammatory response, they are difficult to measure, and circulating TNF concentrations at the time of diagnosis have not been shown to be reliably correlated with disease severity [34]. Circulating IL-6 has been proposed as a marker for a hyperinflammatory response. IL-6, a circulating cytokine induced by TNFα, remains elevated for several days, and its level is correlated with disease severity and can be accurately measured [35, 36, 37].

Previous studies of monoclonal antibody against TNF have looked retrospectively for evidence of a beneficial treatment effect related to baseline plasma IL-6 levels greater than 1000 pg/ml, and data from two large trials did not demonstrate an important treatment effect in patients with these IL-6 levels [22, 32]. Similarly, neither baseline plasma IL-6 or TNF levels are predictive of response to p55 TNF receptor fusion protein [38]. However, in a small study, a retrospective analysis suggested that patients with a baseline IL-6 concentration above 1000 pg/ml derived survival benefit with afelimomab therapy [21].

In conclusion, the predictable pharmacokinetics, lack of overt immune reactions, and few treatment-related adverse events demonstrate that afelimomab is safe and well tolerated. The preliminary analysis of a recently completed randomized placebo-controlled trial ($n = 2634$) indicates that afelimomab treatment of septic patients significantly reduced risk-adjusted mortality (41.5% versus 48.4%, $p = 0.041$, for afelimomab and placebo, respectively) in patients with elevated baseline IL-6 levels.

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Infliximab (chimeric anti-tumour necrosis factor [alpha] monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial

[Articles]

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Summary

Background: Not all patients with rheumatoid arthritis can tolerate or respond to methotrexate, a standard treatment for this disease. There is evidence that antitumour necrosis factor [alpha] (TNF[alpha]) is efficacious in relief of signs and symptoms. We therefore investigated whether infliximab, a chimeric human-mouse anti-TNF[alpha] monoclonal antibody would provide additional clinical benefit to patients who had active rheumatoid arthritis despite receiving methotrexate.

Methods: In an international double-blind placebo-controlled phase III clinical trial, 428 patients who had active rheumatoid arthritis, who had received continuous methotrexate for at least 3 months and at a stable dose for at least 4 weeks, were randomised to placebo (n=88) or one of four regimens of infliximab at weeks 0, 2, and 6. Additional infusions of the same dose were given every 4 or 8 weeks thereafter on a background of a stable dose of methotrexate (median 15 mg/week for >=6 months, range 10-35 mg/wk). Patients were assessed every 4 weeks for 30 weeks.

Findings: At 30 weeks, the American College of Rheumatology (20) response criteria, representing a 20% improvement from baseline, were achieved in 53, 50, 58, and 52% of patients receiving 3 mg/kg every 4 or 8 weeks or 10 mg/kg every 4 or 8 weeks, respectively, compared with 20% of patients receiving placebo plus methotrexate ($p<0.001$ for each of the four infliximab regimens *vs* placebo). A 50% improvement was achieved in 29, 27, 26, and 31% of infliximab plus methotrexate in the same treatment groups, compared with 5% of patients on placebo plus methotrexate ($p<0.001$). Infliximab was well-tolerated; withdrawals for adverse events as well as the occurrence of serious adverse events or serious infections did not exceed those in the placebo group.

Interpretation: During 30 weeks, treatment with infliximab plus methotrexate was more efficacious than methotrexate alone in patients with active rheumatoid arthritis not previously responding to methotrexate.

Introduction

Disease modifying antirheumatic drugs (DMARDS) are useful in the treatment of rheumatoid arthritis. By current consensus, weekly methotrexate has become the standard DMARD in clinical practice,[1,2] either singly or in combination with other DMARDS.[3,4] However, not all patients tolerate these drugs or show an acceptable therapeutic response to them;[5] this has led to

the development of treatment based on a better knowledge of the pathogenesis of rheumatoid arthritis.

There is mounting evidence for a central role of tumour necrosis factor [alpha] in the pathogenesis of rheumatoid arthritis, and tumour necrosis factor [alpha] has emerged as a molecular target for treatment of rheumatoid arthritis.6,7 The first such agent to be assessed in rheumatoid arthritis was a chimeric human-murine monoclonal antibody, a specific inhibitor of tumour necrosis factor [alpha] (infliximab, cA2, Remicade, Centocor Inc).8,9 Several trials have established the efficacy of various anti-tumour necrosis factor agents in relieving symptoms and signs of the disease.10-14 In a recent trial in a small number of patients, infliximab in combination with a fixed low-dose (7.5 mg per week) of methotrexate in rheumatoid arthritis patients with active disease despite methotrexate treatment, showed enhanced degree and duration of efficacy.15 The combination of methotrexate and a tumour necrosis factor-receptor-IgG1 fusion protein (etanercept, Enbrel) is also effective in rheumatoid arthritis patients unresponsive to methotrexate alone.16

This placebo-controlled, double-blind, randomised trial of anti-tumour necrosis factor therapy in patients who were inadequately controlled on methotrexate was undertaken to determine whether infliximab, at two doses every 4 or 8 weeks, added to therapeutic doses of methotrexate, is safe and effective in relief of signs and symptoms of disease.

Methods

Patients

Patients were eligible if they had been diagnosed with rheumatoid arthritis according to the 1987 American College of Rheumatology criteria and had evidence of active disease despite treatment with methotrexate (six or more swollen and tender joints plus two of: morning stiffness greater than or equal to 45 min, erythrocyte sedimentation rate greater than 28 mm/h, C-reactive protein greater than 2 mg/dL. The patients were classified into a functional class (American College of Rheumatology criteria 17). Patients must also have been receiving oral or parenteral methotrexate for at least 3 months with no break in treatment of more than 2 weeks during this period. The methotrexate dose must have been stable at 12.5 mg/week or more, for at least 4 weeks before screening and the patient must have been on a stable dose of folic acid for the same period. Patients using oral corticosteroids (10 mg/kg or less prednisone equivalent) or non-steroidal anti-inflammatory drugs (NSAIDs) must have been on a stable dose for at least 4 weeks before screening: if a patient was not using such drugs, the patient must not have received either drug for at least 4 weeks before screening. The screening laboratory tests must have met the following criteria: haemoglobin 5.3 mmol/L or more, white blood cells 3.5×10^9/L or more, neutrophils 1.5×10^9/L, platelets 100×10^9/L or more, serum aminotransferase and alkaline phosphatase concentration 2 times or less the upper limit of normal, and serum creatinine 150 μmol/L or less.

Patients were excluded if they had little or no ability for self-care; any current inflammatory condition with signs and symptoms that might confound the diagnosis (eg, connective tissue disease or Lyme disease); used a DMARD other than methotrexate or received intraarticular, intramuscular, or intravenous corticosteroids in the 4 weeks before screening; received any other agent to reduce tumour necrosis factor or had any previous use of cyclophosphamide, nitrogen mustard, chlorambucil, or other alkylating agents; or a history of known allergies to murine proteins. Patients were also excluded if they had had infected joint prosthesis during the previous 5 years; serious infections, such as hepatitis, pneumonia, pyelonephritis in the previous 3

months; any chronic infectious disease such as renal infection, chest infection with bronchiectasis or sinusitis; active tuberculosis requiring treatment within the previous 3 years; opportunistic infections such as herpes zoster within the previous 2 months; any evidence of active cytomegalovirus; active *Pneumocystis carinii;* or drug-resistant atypical mycobacterial infection. Other contraindications for inclusion were: current signs or symptoms of severe, progressive, or uncontrolled renal, hepatic, haematological, gastrointestinal, endocrine, pulmonary, cardiac, neurological, or cerebral disease; a history of lymphoproliferative disease including lymphoma or signs suggestive of disease, such as lymphadenopathy of unusual size or location (ie, lymph nodes in the posterior triangle of the neck, infraclavicular epitrochlear, or periaortic areas); splenomegaly; any known malignant disease except basal cell carcinoma currently or in the past 5 years.

After approval of the protocol by the appropriate institutional review boards or ethics committees at all sites, and obtaining informed consent from each patient, enrolment took place at 34 sites in North America and Europe.

Protocol

Figure 1 shows the entry criteria, randomised allocation to five treatment groups, and observation points of the trial protocol. Patients were screened for entrance criteria within 14 days before randomisation. Before the first infusion, patients were assessed for all criteria to provide baseline measurements. Patients were randomly assigned to placebo (0.1% human serum albumin, except in France, where normal saline was used to comply with government regulations) or one of four treatment regimens of infliximab (Remicade, Centocor Inc) at either 3 mg/kg or 10 mg/kg. All patients were given intravenous infusions at weeks 0, 2 and 6. Two infliximab groups (3 mg/kg or 10 mg/kg) and the placebo group received subsequent infusions every 4 weeks, whereas two further groups received infliximab (3 mg/kg or 10 mg/kg) every 8 weeks and placebo infusions on interim 4 week visits to maintain blinding. All infusions were given over 2 hours. Enrolment began on March 31, 1997, and was completed on Jan 23, 1998. An independent organisation did the centralised randomisation. The investigators and patients were blinded to the treatment assignments. An independent assessor, unaware of the patient assignment or other clinical response indices and not involved in the administration of the infusions, assessed the joint scores. An independent clinical research organisation monitored each site. A pharmacist at each site, responsible for study drug preparation, was aware of the assignments.

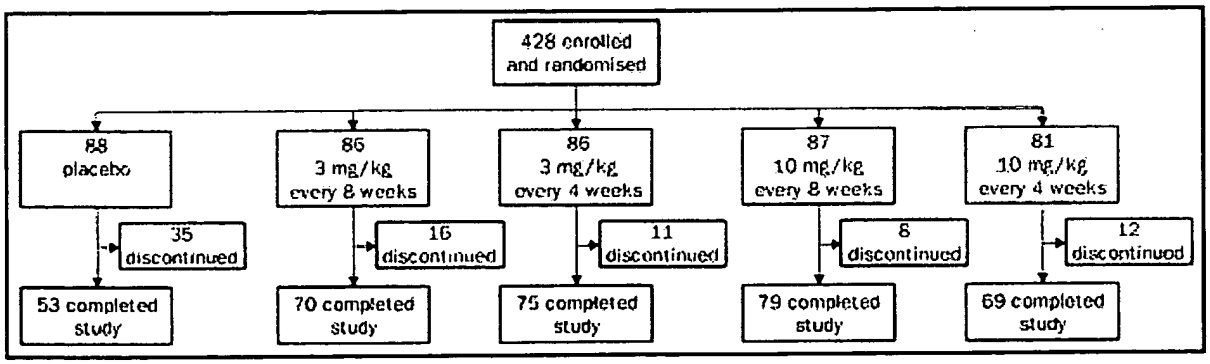

Figure 1: Trial profile

The primary endpoint was prospectively defined as 20% improvement, according to the

American College of Rheumatology [17,18] at the week 30 visit without requiring a surgical joint procedure (ie, arthrodesis and joint replacement); initiation of new drugs for rheumatoid arthritis, or increases in dose of medication for rheumatoid arthritis. Patients received their baseline dose of methotrexate or corticosteroids during the trial. Secondary measurements of response to therapy included documentation of 50% and 70% improvement, reduction in individual measurements of disease activity, and a general health assessment.[19]

Pharmacokinetics and human antichimeric antibody formation

Serum concentrations of infliximab and human antichimeric antibody were measured by a previously described technique.[15] The protocol prespecified that pharmacokinetic data from the first 200 patients would be analysed.

Anti-double strand DNA antibodies and rheumatoid factor

Autoantibodies were measured in two laboratories, one for North American centres and one for European centres. Serum samples for measurement of antinuclear antibodies and antibodies to double-stranded DNA were obtained at baseline and weeks 2, 6, 10, 18, and 26. Antinuclear antibodies were assayed on HEP-2 cells with a starting dilution of 1/40. Serum samples positive for antinuclear antibodies were tested for antibodies to double-stranded DNA by both an immunofluorescence technique on a *Crithidia luciliae* substrate and the Farr assay. A sample was deemed positive for antibodies to double-stranded DNA if a value of greater than or equal to 10 units/mL was obtained by the Farr assay and was also positive by the *Crithidia* immunofluorescent test. Rheumatoid factor was measured by nephelometry at baseline, 10 weeks and 30 weeks (normal range 0-39 IU/mL and 0-12 IU/mL, respectively, for North American and European central laboratories).

Statistical analyses

Patients were assigned to a treatment group with an adaptive stratified randomisation, with investigational sites as the strata. The sample size of about 80 patients per treatment group provided more than 90% power to detect a difference in proportions between treatment groups by use of the two-sided $[chi]^2$ test at [alpha]=0.01, where the methotrexate-alone group was predicted to have a 20% clinical response rate and the infliximab group with the highest response was predicted to have a 65% clinical response rate (the remaining infliximab treatment groups were predicted to be midway between these two extremes). All 428 patients who satisfied entry criteria were randomised to treatment, and all received at least one infusion of study drug. Efficacy analyses and analyses of baseline characteristics used the randomised patient groups. An intention-to-treat analysis was done for the primary efficacy endpoint (the American College of Rheumatology 20% response at the 30 week visit). Analyses of safety endpoints were undertaken with patients grouped as treated; three patients were reclassified from their randomised treatment groups in the safety analyses as described below. As a result of errors, two placebo patients each received one infusion of infliximab and were analysed for safety indices in the 3 mg/kg every 8 weeks group; one further patient who was randomised to the 10 mg/kg every 4 weeks group actually received 3 mg/kg every 8 weeks for most of the 30 weeks and was analysed for safety as if randomised to this dose. Two sensitivity analyses assessed the robustness of the primary efficacy results: first, by analysis after classifying of patients as nonresponders if they discontinued study treatment because of a lack of efficacy or an adverse event; and second by repeating the primary efficacy analysis with actual results at week 30 for patients who had surgical joint procedures or an increase in their drugs for rheumatoid arthritis.

All statistical testing began with a test for an overall treatment effect across the five treatment groups. Categorical variables were tested by the [chi]² test, and continuous variables were tested by analysis of variance on the van der Waerden normal scores. Pairwise analyses comparing infliximab treated groups with the placebo group were only done if the test for an overall treatment effect was significant ($p<0.05$). Pairwise testing of categorical efficacy endpoints used the [chi]² test, and pairwise testing of categorical safety endpoints used Fisher's exact test. For continuous variables, pairwise comparisons of infliximab groups versus placebo were made with linear contrasts. All statistical testing was two-sided.

Results

Characteristics of randomised population

The baseline characteristics of the five treatment groups were well matched (table 1), and consisted of a predominantly white, female, rheumatoid factor-positive population, with a median age range of 51-56 years, and disease duration of 7.2 to 9.0 years. About half the patients were in functional class III and three of 428 in functional class IV. In the treatment groups a mean of 2.5-2.8 DMARDS (excluding methotrexate) had been used and included drugs such as gold (237 patients, 63%), sulphasalazine (217 patients, 58%). hydroxychloroquine (202 patients, 54%), chloroquine (53 patients, 14%), d-penicillamine (89 patients 24%), azathioprine (76 patients, 20%), and cyclosporine (70 patients, 19%). 27-41 patients (31-48%) had had joint surgery. There was a considerable level of disease activity at baseline, despite treatment with methotrexate and other concomitant drugs (table 2). The recalcitrant nature of the disease was shown by an inadequate response to a median dose of methotrexate at entry (15 [range 10-35] mg/week; 72% of patients receiving >=15 mg/week), stable for at least 1 month, but, on analysis, this median dose proved identical to that received within the previous 6 months. Overall 366 patients (86%) had received methotrexate for more than a year and more than half the patients had received methotrexate for 3 or more years, with an estimated cumulative dose of 3000 mg in 30% of patients. 259 (61%) patients entered the trial on stable doses of corticosteroids and 318 (74%) entered on stable doses of NSAIDs.

	Placebo (n=88)	Infliximab			
		3 mg/kg every 8 wk (n=86)	3 mg/kg every 4 wk (n=86)	10 mg/kg every 8 wk (n=87)	10 mg/kg every 4 wk (n=81)
Demographics					
Age (years) median (range)	51 (19·0, 75·0)	56 (25·0, 74·0)	51 (19·0, 78·0)	55 (19·0, 80·0)	52 (23·0, 74·0)
Female (%)	70 (80)	70 (81)	66 (77)	67 (77)	59 (73)
Race (white %)	78 (89)	80 (93)	76 (88)	79 (91)	76 (94)
Disease status					
Duration (years) median (range)	8·9 (0·8, 35·0)	8·4 (0·7, 45·0)	7·2 (0·5, 33·8)	9·0 (0·5, 49·9)	8·7 (0·6, 47·0)
Rheumatoid factor positive (%)	67 (77)	72 (84)	69 (80)	71 (82)	66 (82)
Functional class III or IV) %†	40 (45)	44 (51)	41 (48)	47 (54)	39 (48)
Previous joint surgery (%)	29 (33)	41 (48)	33 (38)	27 (31)	30 (37)
Synovectomy	10 (11)	17 (20)	12 (14)	12 (14)	12 (15)
Arthrodesis	10 (11)	16 (19)	13 (15)	10 (12)	8 (10)
Joint replacement	17 (19)	25 (29)	21 (24)	16 (18)	20 (25)
Drug treatments					
Receiving NSAIDs (%)	63 (72)	68 (79)	65 (76)	67 (77)	55 (68)
Receiving corticosteroids (%)	56 (64)	54 (63)	48 (53)	50 (57)	53 (65)
Mean no (SD) of previous DMARDs (excluding methotrexate)	2·5 (1·4)	2·8 (1·5)	2·6 (1·5)	2·5 (1·4)	2·5 (1·3)
Methotrexate (mg/wk)	15	15	15	15	15
IQR‡	12·5–17·5	12·5–17·5	12·5–17·5	12·5–17·5	15·0–20·0
Duration of methotrexate					
<1 year (%)	12 (14)	15 (17)	15 (17)	10 (11)	10 (12)
1–3 years (%)	32 (36)	29 (34)	31 (36)	30 (35)	26 (32)
>3 years (%)	44 (50)	42 (49)	40 (47)	47 (54)	45 (56)

*Median values throughout unless otherwise stated; no significant differences between infliximab and placebo treated groups for any index. †Three patients in class IV. ‡IQR=interquartile range.

Table 1: Patient characteristics

	Placebo		Infliximab								% change from baseline at 30 weeks†				
			3 mg/kg every 8 wk		3 mg/kg every 4 wk		10 mg/kg every 8 wk		10 mg/kg every 4 wk		Placebo	3 mg/kg every 8 wk	3 mg/kg every 4 wk	10 mg/kg every 8 wk	10 mg/kg every 4 wk
Week	0	30	0	30	0	30	0	30	0	30					
Swollen joint count (0–66)	19	13	19	9	20	9	20	7	23	6	20	-52	-50	-69	-64
IQR	13, 28	8, 25	13, 30	4, 18	12, 29	4, 15	13, 29	4, 15	15, 31	3, 12					
p value	—		<0·001		<0·001		<0·001		<0·001			<0·001	<0·001	<0·001	<0·001
Tender joint count range (0–68)	24	18	27	12	31	11	30	12	35	9	-28	-59	-65	-58	-65
IQR	16, 46	7, 33	16, 46	3, 31	20, 39	4, 25	20, 44	4, 22	22, 44	2, 25					
p value	—		0·032		0·031		0·012		0·002			0·006	0·030	<0·001	<0·001
Pain score range (VAS 0–10 cm)§	6·7	5·9	7·0	3·5	6·9	3·5	6·7	3·1	6·6	3·7	-6	-33	-43	-50	-38
IQR	5·0, 8·0	3·3, 7·4	5·6, 8·1	2·3, 6·0	4·8, 7·8	1·2, 6·2	5·8, 7·6	1·2, 5·7	5·0, 7·7	1·1, 5·6					
p value	—		0·075		<0·001		<0·001		<0·001			0·026	<0·001	<0·001	<0·001
Evaluator's global score range (VAS 0–10 cm)§	6·5	5·0	6·1	2·6	6·2	2·6	6·4	2·6	6·0	2·5	-13	-53	-59	-58	-59
IQR	5·2, 7·4	3·0, 7·0	4·8, 7·1	1·5, 5·7	4·6, 7·3	0·8, 4·6	5·0, 7·1	1·3, 4·3	4·6, 7·0	1·2, 4·2					
p value	—		<0·001		<0·001		<0·001		<0·001			<0·001	<0·001	<0·001	<0·001
Patient's global score range (VAS 0–10 cm)§	6·2	5·5	6·0	3·0	5·7	3·0	6·4	3·7	6·0	3·3	-7	-23	-30	-40	-47
IQR	4·3, 8·1	3·1, 7·5	4·9, 7·8	1·6, 6·7	4·3, 8·0	1·5, 6·0	5·0, 7·7	1·3, 6·5	4·6, 7·7	1·1, 5·9					
p value	—		0·041		0·003		<0·001		<0·001			0·008	0·002	<0·001	<0·001
Health assessment questionnaire (0–3)¶	1·5	1·5	1·6	1·5	1·6	1·1	1·6	1·4	1·5	1·3	-3	-13	-29	-27	-24
IQR	1·3, 2·1	1·0, 2·3	1·4, 2·3	0·9, 2·1	1·3, 2·1	0·6, 1·9	1·3, 2·1	0·5, 1·8	1·3, 2·1	0·5, 1·8					
p value	—		0·765		0·017		0·002		0·002			0·167	<0·001	<0·001	<0·001
C-reactive protein concentration (mg/dL)	3·0	2·3	3·1	0·8	3·0	0·5	2·5	0·6	2·4	0·5	-9	-60	-61	-68	-76
IQR	1·2, 5·7	0·7, 5·1	1·2, 5·2	0·4, 2·3	0·8, 4·4	0·3, 1·4	1·0, 4·0	0·3, 1·3	1·1, 5·3	0·3, 1·1					
p value	—		<0·001		<0·001		<0·001		<0·001			<0·001	<0·001	<0·001	<0·001
Rheumatoid factor concentration (U/mL)	188	172	143	98	231	117	178	75	177	111	0	-37	-27	-46	-31
IQR	34, 459	45, 462	44, 315	23, 192	65, 491	31, 312	48, 429	27, 209	60, 413	34, 294					
p	..	..	..	..	..	..	..	..	..	..	..	<0·001	<0·001	<0·001	<0·001

*Median values; †the percent reduction achieved at 30 weeks relative to baseline is calculated for every patient. Percentage change from baseline shown in the table is the median of these values in each treatment group. ‡p value vs placebo, χ² test; §VAS=visual analogue scale; ¶0=no discomfort or disability, 3=maximum disability. IQR=interquartile range.

Table 2: Individual clinical and laboratory assessments of disease activity at baseline (week 0) and 30 weeks* and % change from baseline†

Discontinuations

35 of 88 (36%) patients in the placebo group discontinued, a number that significantly exceeded those discontinuing infliximab (8-16 patients 9-18%). Lack of efficacy accounted for withdrawal of 22 patients in the placebo and between five and 11 patients in the infliximab groups. Similar numbers of patients in the placebo or infliximab group discontinued therapy because of adverse events (seven patients; 8% placebo, and three to six patients 3-7% infliximab). Two patients on placebo were withdrawn for non-compliance, and withdrawal of consent accounted for discontinuation in one patient in each of the placebo and 3 mg/kg every 8 weeks, and 10 mg/kg every 8 weeks infliximab groups. Three patients in the placebo group and two in the infliximab treated groups died during the 30 week trial (see results section for details).

Efficacy

Infliximab proved efficacious as judged by all the response criteria used. More patients ($p<0.001$) treated with infliximab, achieved the intention-to-treat primary efficacy measurement (the American College of Rheumatology 20 response) than in the placebo group (figure 2). The odds ratios (95% CI) were 3.9 (2.0-7.6), 3.9 (2.0-7.6), 4.2 (2.1-8.1), and 5.4 (2.7-10.6) for the 3 mg/kg every 8 weeks, 3 mg/kg every 4 weeks, 10 mg/kg every 8 weeks, and 10 mg/kg every 4 weeks, respectively.

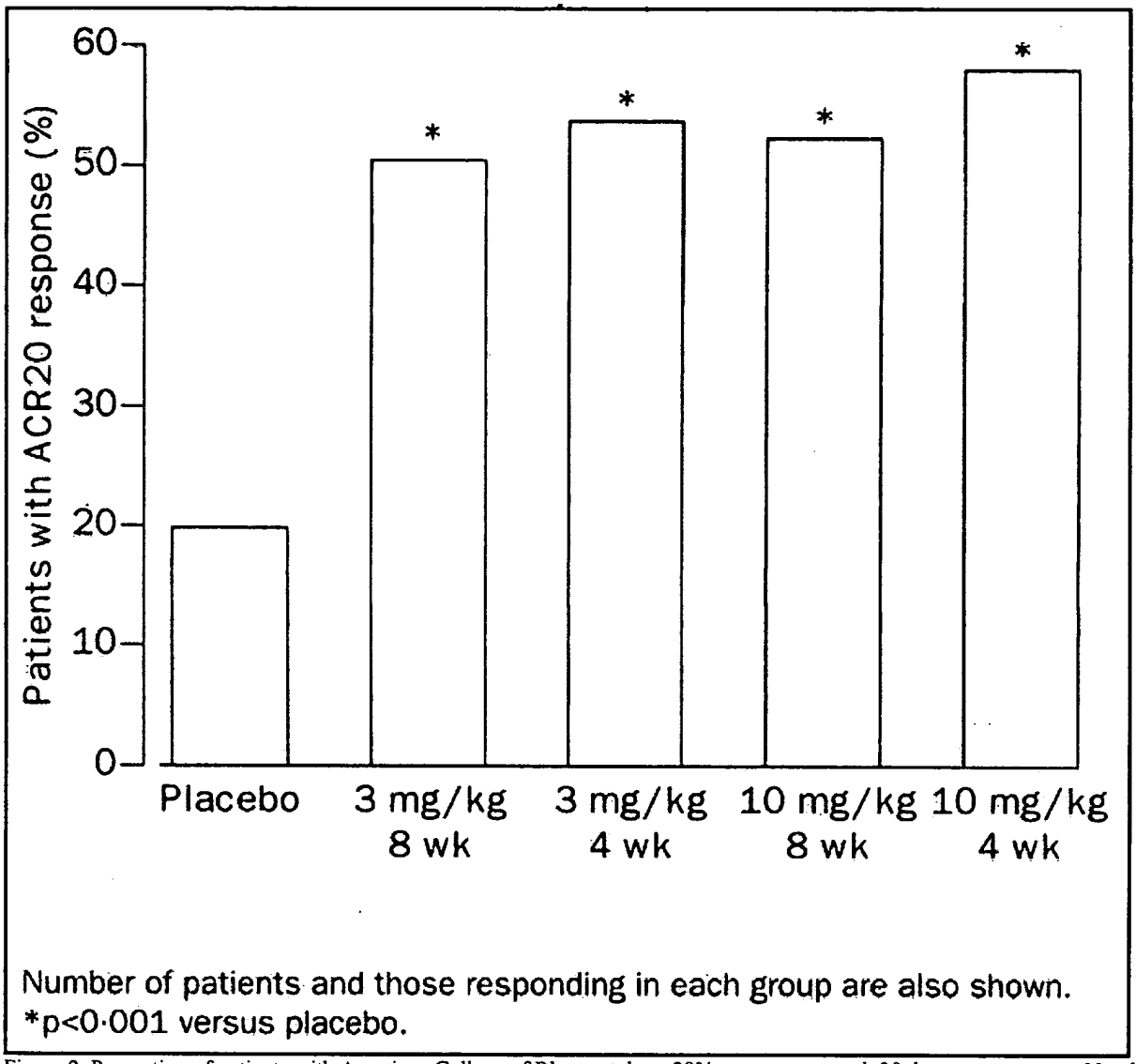

Figure 2: Proportion of patients with American College of Rheumatology 20% response at week 30, by treatment groupNumber of patients and those responding in each group are also shown. *p<0.001 versus placebo.

The number of patients classified as non-responders because of changes in drug were: seven in the placebo group, two each in the 3 mg/kg every 4 and 8 weeks treatment groups; two in the 10 mg/kg every 8 weeks and 4 in the 10 mg/kg every 4 weeks treatment groups. A significant response ($p<0.001$) was seen even when groups of responders and non-responders were reclassified as described in the statistical analysis section, thus demonstrating the robustness of the primary efficacy analyses.

In all groups, response was rapid, with over half the ultimate responders, attaining the American College of Rheumatology 20% response by the first evaluation at 2 weeks, and about 90% at the 6 week evaluation (figure 3[a]). Thereafter, following a further small increment, total response rates were sustained at levels between 50% and 60% up to the 30 week endpoint.

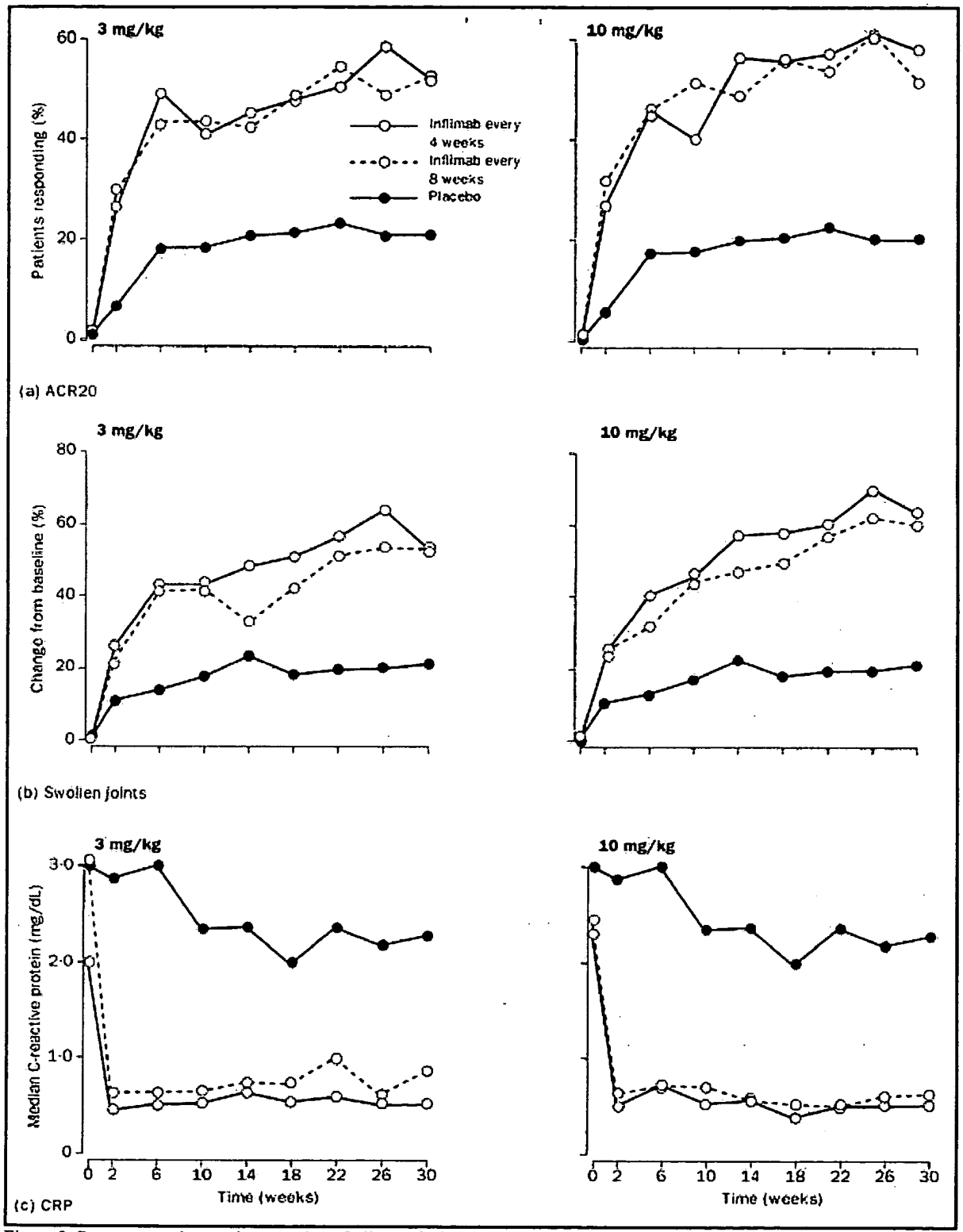

Figure 3: Response to therapy by American College of Rheumatology (ACR) criteriaImprovement in swollen joint count (defined in table 2); and serum C-reactive protein (CRP) concentration (swollen joints and C-reactive protein graphs use last-observation-carried-forward data).

A significantly greater percentage of infliximab-treated than placebo-treated patients achieved more substantial responses defined by American College of Rheumatology-50 and 70 criteria (table 3). Improvement in each individual clinical measurement of disease activity was

consistently recorded in all infliximab groups (table 2). To assess the magnitude of individual clinical responses we calculated the percentage reduction at 30 weeks from baseline. For several indices the values showed a trend towards improvement in placebo-treated patients, but these changes were small relative to the improvement recorded with infliximab (table 2). Particularly noteworthy, the median titre of rheumatoid factor fell in the infliximab treated groups with no change in the placebo group ($p<0.001$) When interpreting table 2, it should be kept in mind that the percentage change from baseline at 30 weeks presents the median of the change from baseline calculated for each individual patient; this may differ from the median result of individual measurements in each treatment group at baseline with the median result at 30 weeks. The clinical improvement in infliximab treated patients occurred rapidly (figure 3). The data between 6 and 30 weeks for swollen joint counts show greater continuing improvement (figure 3 [b]) than for American College of Rheumatology 20 and C-reactive protein measurements (figure 3 [a] and [c]).

Response	Placebo+ methotrexate (n=84)	Infliximab plus methorexate			
		3 mg/kg every 8 wk (n=83)	3 mg/kg every 4 wk (n=85)	10 mg/kg every 8 wk (n=85)	10 mg/kg every 4 wk (n=80)
ACR 50	4 (5%)	22 (27%)	25 (29%)	26 (31%)	21 (26%)
p	..	<0·001	<0·001	<0·001	<0·001
ACR 70	0 (0%)	7 (8%)	9 (11%)	15 (18%)	9 (11%)
p	..	0·007	0·002)	<0·001	0·002

*ACR 50 and 70 respectively refer to the level of response to therapy which achieved 50% or 70% change from baseline in the swollen and tender joint counts plus at least 3 of 5 other measurements of disease activity according to the American College of Rheumatology criteria.[13]

Table 3: Patients achieving ACR* 50% and 70% response at 30 weeks

Pharmacokinetics and human antichimeric antibody formation

Pharmacokinetic measurements were made in 197 patients. Before the scheduled infusion at 30 weeks, the mean (SD) serum concentration of infliximab at the 3 mg/kg every 8 or 4 weeks doses was 1.5 (1.6) µg/mL and 9.7 (8.6) µg/mL; and at the 10 mg/kg every 8 or 4 weeks was 8.9 (8.1) µg/mL and 35.8 (23.7) µg/mL, respectively. The previously defined half-life of infliximab was 8-12 days, consistent with mainly an intravascular distribution. Human antichimeric antibody formation could not be measured in most patients receiving infliximab because serum infliximab interferes with the assay. However, of 27 infliximab-treated patients who discontinued treatment before 30 weeks, three tested positive for human antichimeric antibodies (two with a titre of 1/10 and one with a titre of 1/40).

Safety

The eight infliximab infusions were generally well tolerated. Adverse experiences (table 4) were common in all groups, including placebo, and were reported at least once in over 80% of patients. Upper respiratory tract infections and headache were the most commonly seen adverse

events. 14-16 patients (16-20%) receiving infliximab, compared with nine (10%) treated with placebo (p=0.477) developed an infusion reaction, defined as any adverse experience occurring during, or up to 1 h after completion of, the infusion. The frequency of infusion reactions was highest with the first infusion. Most of the infusion reactions were mild (headache and nausea being most common) and transient. Symptoms were controlled by slowing down the infusion rate or the prophylatic use of antihistamines (the latter in less than 10% of patients), or both. Only two patients on infliximab (of 340) discontinued treatment because of an infusion reaction (one urticaria, one dyspnoea), and no infusion reactions were classified as serious. Adverse events suggestive of an immediate hypersensitivity reaction were infrequent and generally mild (hypotension: eight patients, 2.3% infliximab *vs* placebo two patients, 2.3%; urticaria: four patients, 1.2% for infliximab *vs* no patients on placebo; dyspnoea: two patients, 0.6% for infliximab *vs* no patients on placebo). In this study no delayed hypersensitivity reactions were reported 1 hour after the completion of infusion and the next observed time-point 4 weeks later.

	Placebo	Infliximab			
		3 mg/kg every 8 wk	3 mg/kg every 4 wk	10 mg/kg every 8 wk	10 mg/kg every 4 wk
Patients treated	86	89	86	87	80
Upper respiratory tract infection	14 (16%)	29 (33%)	17 (20%)	21 (24%)	18 (23%)
Headache	9 (10%)	22 (25%)	17 (20%)	21 (24%)	16 (20%)
Nausea	16 (19%)	14 (16%)	12 (14%)	12 (14%)	14 (18%)
Sinusitis	4 (5%)	10 (11%)	6 (7%)	12 (14%)	12 (15%)
Rash	4 (5%)	5 (6%)	7 (8%)	14 (16%)	11 (14%)
Coughing	3 (3%)	8 (9%)	6 (7%)	11 (13%)	12 (15%)
Diarrhoea	10 (12%)	7 (8%)	8 (9%)	7 (8%)	10 (13%)
Fatigue	6 (7%)	15 (17%)	5 (6%)	3 (3%)	9 (11%)
Dizziness	6 (7%)	8 (9%)	5 (6%)	12 (14%)	5 (6%)
Rhinitis	5 (6%)	7 (8%)	5 (6%)	10 (11%)	7 (9%)
Back pain	2 (2%)	7 (8%)	7 (8%)	6 (7%)	8 (10%)
Abdominal pain	7 (8%)	4 (4%)	8 (9%)	7 (8%)	6 (8%)
Pain	4 (5%)	4 (4%)	3 (3%)	7 (8%)	8 (10%)
Pharyngitis	4 (5%)	5 (6%)	4 (5%)	6 (7%)	6 (8%)
Arthralgia	2 (2%)	6 (7%)	2 (2%)	5 (6%)	5 (6%)
Hypertension	3 (3%)	5 (6%)	3 (3%)	4 (5%)	6 (8%)
Stomatitis, ulcerative	2 (2%)	4 (4%)	3 (3%)	2 (2%)	9 (11%)
Urinary tract infection	3 (3%)	3 (3%)	2 (2%)	6 (7%)	7 (9%)
Fever	4 (5%)	4 (4%)	7 (8%)	3 (3%)	4 (5%)
Dyspepsia	3 (3%)	5 (6%)	5 (6%)	1 (1%)	6 (8%)
Any infection	34 (40%)	47 (53%)	40 (47%)	56 (64%)	58 (73%)
Infection requiring antimicrobials	18 (21%)	20 (23%)	24 (28%)	32 (37%)	30 (38%)
Serious infections	5 (6%)	1 (1%)	5 (6%)	5 (6%)	3 (4%)
Serious adverse events	14 (16%)	8 (9%)	11 (13%)	8 (9%)	10 (13%)

Table 4: Adverse events

The number of serious adverse events (requiring hospitalisation or judged life threatening) was no higher in the infliximab group (8-11 patients; 9-13%), than in those receiving placebo, 14 patients (16%). The frequency of any infection was significantly increased in patients receiving 10 mg/kg of infliximab, but not in those receiving 3 mg/kg (table 4). The number of infections classified as serious (life threatening or leading to hospital treatment) was no more frequent with infliximab one to five patients (1-6%) than in five patients receiving placebo (6%).

After a total of 445 patient-years of follow-up, representing 86 patient-years of placebo and 359 patient-years of infliximab, three patients were reported to have malignant disease, all

occurring in those on infliximab 10 mg/kg every 4 weeks. These diseases were: recurrence of carcinoma of the breast (one patient); squamous cell carcinoma and melanoma (one patient); and B cell lymphoma (one patient). However, the observed incidence was not higher than the 2.8 cases expected based on an age-matched sex-control population from the Surveillance Epidemiology and End Results database of National Institutes of Health (USA).20

Three of 88 patients in the placebo group (3%) and two of 340 receiving infliximab (0.6%) died in the 30 weeks. The three deaths in the placebo group were due to (i) pneumonia, sepsis, intestinal gangrene, and cardiopulmonary failure complicating rheumatoid arthritis; (ii) interstitial lung disease, heart failure, and pericardial effusion associated with active rheumatoid arthritis; and (iii) ischaemic and necrotic liver and bowel leading to cardiopulmonary failure. In the infliximab group, deaths were due to (i) cardiopulmonary failure as a result of suspected pulmonary embolism or interstitial lung disease, after a successfully treated episode of bacteraemia and septic arthritis 2 months before death, and whilst receiving heparin prophylaxis; and (ii) pulmonary embolism secondary to venous thrombosis in a patient with history of ischaemic heart disease and myocardial infarction. Both patients had been randomised to 3 mg/kg regimens, the former having received a total of two, and the latter three, infusions of infliximab.

Abnormalities in liver function tests, (serum aspartate transaminase, alanine transaminase, alkaline phosphatase and bilirubin) of more than twice the normal range occurred in all groups with no significant differences between infliximab and placebo groups. One patient receiving infliximab 3 mg/kg every 8 weeks developed dyspnoea suspected to be due to methotrexate toxicity, and discontinued study treatment. One patient in the placebo group discontinued because of iron deficiency anaemia and a second on placebo because of thrombocytopenia. No other serious haematological abnormalities were recorded.

Antibodies to double-strand DNA have been previously reported in infliximab treated patients 11,15 and in this trial, with a cut-off in the Farr assay of 10 units/mL (and a positive crithidia test), 54 patients (16%) were positive (*vs* none in the placebo group). Only 15 patients (4%) had a titre of 25 units/mL or higher during the trial. None of the patients were positive for antibodies to double-strand DNA at baseline. No dose-response relation was seen. One of 340 infliximab treated patients was diagnosed as having a drug-induced lupus syndrome after two treatments with infliximab 10 mg/kg, characterised by a rash on the face, hands, and forearms with an antinuclear antibody titre rising from 1/40 at baseline to 1/80, associated with a low complement C4, but without developing antibodies to double-strand DNA. The patient discontinued treatment and showed recovery from the rash at follow-up.

Discussion

This placebo-controlled trial provides evidence for a rapid reduction in disease activity measurements in response to infliximab at 3 mg/kg and 10 mg/kg in patients inadequately controlled with therapeutic doses of methotrexate, with a significant improvement in over half the treated patients. Maintenance treatment with infliximab 3 mg/kg every 8 weeks (the lowest dose used) shows that the response is sustained for up to 30 weeks with equivalent efficacy to 3 mg/kg every 4 weeks and 10 mg/kg every 4 or 8 weeks. Discontinuation due to lack of efficacy was lower in patients receiving infliximab at all doses than in patients on placebo. Moreover, the dropout rate due to adverse events did not exceed that in the placebo group, and in the 3 mg/kg group every 8 weeks was less than half that recorded in the placebo group.

The analysis thus provides support for up to 30 weeks of treatment of rheumatoid arthritis by combining infliximab with methotrexate without an increase in serious adverse events. The response and safety characteristics in those receiving infliximab every 8 weeks offers advantages in terms of cost, convenience, and compliance over 4-weekly regimens.

The degree of improvement (50-58%) is particularly noteworthy in view of the inclusion of patients resistant to at least three DMARDS (including methotrexate) and with aggressive disease. Three deaths in the 88 placebo-treated patients during the 6 months further supported the serious outlook of the patients in this study. Investigation of patients with earlier disease, and those without irreversible joint damage in whom disease activity is easier to measure than in this study, may show that this anti-tumour necrosis factor [alpha] therapy is effective in a greater proportion of patients unresponsive to methotrexate.

No difference in the number of serious adverse events was noted among infliximab and placebo treated patients. Nevertheless, experimental data suggest that tumour necrosis factor [alpha] can lead to inflammation and modifies cellular immune response, and the theoretical possibility exists that anti-tumour necrosis factor may alter immune responses to infection. During the 30 week trial there was no increase in serious infections requiring hospital treatment, but infections were more common in the 10 mg/kg regimens of infliximab than in the other treatment groups. In the continuing second 6 months of the trial one patient has developed tuberculosis and one coccidiomycosis (both on infliximab). The patient with tuberculosis was randomised to infliximab 3 mg/kg every 4 weeks and was receiving methotrexate 12.5 mg weekly. After a protracted illness diagnosed as disseminated tuberculosis and treated with antituberculous drugs (the organism was later found to be resistant to isoniazid, rifampicin, pyrazinamide, and clarithromycin) the patient died. The patient with coccidiomycosis had been randomised to infliximab 10 mg/kg every 8 weeks and received infliximab for 9 months and concomitant methotrexate 15 mg/week along with corticosteroid therapy. Despite amphotericin for 1 month the patient died. The extent to which infliximab contributed to the mycobacterial and fungal infection in these two patients and the assessment of future risk will require surveillance of a larger cohort of patients. Meanwhile it would be prudent to avoid (or withdraw) infliximab therapy in patients with severe acute, chronic, or recurrent infections, and especially to bear in mind the possibility of mycobacterial and fungal infection in cases of pyrexia of uncertain aetiology.

The number of infusion reactions among patients on infliximab was low and did not increase with repeated infusions. Transient reactions were graded as non-serious and generally of mild to moderate intensity and only two of 340 patients on infliximab were regarded as a limitation to continuing treatment. Taken together with the stable pharmacokinetics and low number of human antichimeric antibody, the data are consistent with the low immunogenicity of the chimeric antibody in this trial.

In previous trials infliximab induced antibodies to double-strand DNA in a few rheumatoid arthritis patients and these seemed non-pathogenic in the context of continuing infliximab treatment, with only one patient (of about 220 patients) developing a drug-induced lupus syndrome that resolved after cessation of therapy.[15] In this trial lasting over 7 months in 342 exposed patients, induction of low levels of antibodies to double-strand DNA was seen in up to 54 (16%) of patients. However, if a clinically more relevant positive result in the Farr assay (>=25 U/mL) was used, only 4% of patients (15) developed the antibody. Whether the development of antibodies to double-strand DNA predicts the onset of drug-induced lupus is unknown, since the sole such patient in this trial was anti-double-strand DNA negative. Double-strand DNA antibodies at similar frequencies to those seen in infliximab trials have been

reported in patients receiving etanercept.[14] Since rheumatoid factor has been implicated in the pathogenesis of rheumatoid arthritis, the reduction of this autoantibody after therapy is in striking contrast to the induction of antibodies to double-strand DNA. In previous trials we have investigated the occurrence of cardiolipin antibodies and found no consistent change associated with infliximab treatment.

In this trial, four cancers (three of epithelial cell origin and one a lymphoma) were reported in three infliximab treated patients in 359 patient-years of follow-up. On the basis of age-and-sex-specific occurrence of cancer in the general population,[20] the expected incidence would be 2.8 cases in this period of observation, which is similar to that in the trial. One cancer was a non-Hodgkin's B cell lymphoma. Three lymphomas (two non-Hodgkin's and one Hodgkin's) have been recorded in six clinical trials in a total of 555 rheumatoid arthritis patients treated with infliximab and followed for 6 months to 3 years. This experience has to be viewed against a background of several reports of an increase in B cell lymphomas in rheumatoid arthritis, especially with previous exposure to azathioprine and methotrexate.[21,22] Furthermore, a Swedish nested, case-control study showed an increased rate of lymphoma in rheumatoid arthritis patients with high grade inflammatory arthritis of the type included in this study, with an odds ratio of 25.8.[23]

Our findings are comparable overall with those of Weinblatt and colleagues [16] with etanercept and methotrexate, which is not surprising since both infliximab and etanercept exert their effects by the blockade of overproduction of tumour necrosis factor [alpha] in rheumatoid arthritis. In the absence of a head-to-head trial, detailed comparisons of these two studies would not be informative. However, there are important differences between the studies, for example this study was conducted at 34 international centres compared with seven centres in the US for the etanercept study, and enrolled five times as many patients, possibly with more advanced disease. It is noteworthy that the placebo response rate in the ATTRACT (infliximab) study was lower than in the etanercept study. Since infliximab is given as boluses of intravenous injections every 4 or 8 weeks, the pharmacokinetics are likely to be different from twice weekly subcutaneous etanercept.

This clinical trial indicates an acceptable safety profile relative to the benefit provided. The 6 month safety followup data are reassuring, but longer term treatment and surveillance are still needed, particularly with respect to the occurrence of serious infectious complications and lymphoma with infliximab. The cost-effectiveness of antitumour necrosis factor therapy must also await longer term data evaluating the cost of this therapy and the improvement in the quality of life that might follow, set against the cumulative social and health care costs of current therapies.

Contributors

The paper was written primarily by Ravinder Maini with the assistance of the other authors. All authors contributed to the design, implementation of the study and approving the final version of the manuscript.

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